REMARKS/ARGUMENTS

In response to the Office Action of February 4, 2004, Applicants request re-examination and reconsideration of this application for patent pursuant to 35 U.S.C. 132.

Claim Status/Support for Amendments

Claims 1, 39 and 44-46 have been amended. Claims 2-38 were cancelled in a previous reply (filed on October 23, 2003). Claims 39-46 are withdrawn from consideration. It is understood that the remaining claims 39-46, drawn to the non-elected invention, will remain pending, albeit withdrawn from prosecution on the merits at this time. Claim 1 is under examination. Claims 1 and 39-46 remain pending in the instant application.

No new matter has been added by the amendments to the specification made herein.

The title of the invention has been amended to correct a punctuation error in the reciting of Alzheimer's disease (Alzheimers corrected to Alzheimer's).

In the "Background of the Invention" section a punctuation error was corrected at page 1, line 23.

The disclosure of prior art, PCT/EP97/04396, at page 5 has been amended to correct a typographical error in the international application number. The corresponding international publication

number has also been added.

The "Description of the Figures" section has been amended to add sequence identification numbers, clearly indicate that Figures 2, 4 and 6 show the mass spectrum profiles of the disclosed peptides, to correct a punctuation error in the reciting of Alzheimer's disease (Alzheimers corrected to Alzheimer's) and to properly identify a trademark name (SEPHAROSE).

Several protocols at pages 41-45 have been amended to properly identify trademark names (SEPHAROSE, TRITON, TRIS and EPPENDORF). The protocol titles at page 41 (line 13), page 42 (lines 4 and 19), and page 43 (lines 10 and 23) were underlined in the original disclosure and do not indicate amended text.

The paragraph at page 46 was amended to correct a punctuation error in the reciting of Alzheimer's disease (Alzheimers corrected to Alzheimer's).

In the "Detailed Description" section, the term "cerebrospinal fluid" has been added to define the abbreviation "CSF" at page 49, line 17 in order to provide explicit support for cerebrospinal fluid as recited in claim 41. "CSF" is a well-known abbreviation for cerebrospinal fluid in the biochemical art. A typographical error within the same paragraph has also been amended (skill replaced skilled).

No new matter has been added by the amendments to the claims made herein.

Claim 1 has been amended to specifically claim the biopolymer marker (amino acid residues 2-18 of SEQ ID NO:1). The term "biopolymer marker" is used throughout the originally filed specification, see, for example, page 1, line 8.

Claim 39 has been amended to more clearly disclose the relationship between the presence of the claimed biopolymer marker (amino acid residues 2-18 of SEQ ID NO:1) and Alzheimer's disease. Claim 39 has also been amended to clearly indicate how the presence of the claimed biopolymer marker is determined from mass spectrum profiles. The changes to claim 39 find basis throughout the original disclosure, see, for example, page 35, lines 14-18, page 46, lines 11-19 and Figures 1 and 2.

Claim 44 has been amended to correspond with the biopolymer marker of claim 1, as amended herein. Support for various types of kits can be found in the original disclosure, see, for example, page 36, lines 9-12 and page 47, line 15 to page 49, line 1.

Claims 45 and 46 were amended to provide proper antecedent basis for the term "kit" in claim 44, as amended herein.

As used herein the term "normal patient" refers to a patient who is normal with regard to Alzheimer's disease, in other words, such normal patient has not been diagnosed with Alzheimer's disease, but may or may not have other conditions.

Oath/Declaration

Applicants note that while the original oath filed on March 13, 2002 contains the signature of Dr. John Marshall (inventor 2), the date of signature is omitted.

Applicants are currently in the process of preparing a new oath and will forward such oath to the Examiner as soon as it is completed and properly executed.

Restriction

The Examiner has withdrawn claims 39-46 from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a non-elected invention(s), there being no allowable generic or linking claim.

New claims 39-46 were added in the Reply to the Requirement for Restriction filed on June 9, 2003.

The Examiner refers to MPEP 821.03 regarding the election of claims by original presentation. Form paragraph 8.04 of MPEP 821.03 indicates that an invention is constructively elected by original presentation for prosecution on the merits following an action **on the merits**. Emphasis added by Applicant.

Request for Rejoining of Claims

Considering that claims 39-46 are limited to the use of an isolated biopolymer marker consisting of amino acid residues 2-18

of SEQ ID NO:1, a search of these claims would encompass this specific biopolymer marker. The instant application is related in claim format to several other applications, both pending and issued, of which serial number 09/846,352 is exemplary. In an effort to maintain equivalent scope in all of these applications, Applicants respectfully request that the Examiner consider rejoining claims 39-46 in the instant application, which are currently drawn to non-elected inventions, under the decision in *In re Ochiai* (MPEP 2116.01) with claim 1 of the elected invention, upon the Examiner's determination that the claim of the elected invention is allowable and in light of the overlapping search. If the biopolymer marker consisting of amino acid residues 2-18 of SEQ ID NO:1 is found to be novel, methods and kits limited to its use should also be found novel.

Rejection under 35 USC 112

Claim 1, as presented on October 23, 2003, stands rejected under 35 USC 112, first paragraph, as containing subject matter which allegedly was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner continues to maintain the position that the instant specification, as filed, fails to provide any evidence or

sound scientific reasoning that would support the conclusion that the presence of an isolated peptide consisting of amino acid residues 2-18 of SEQ ID NO:1 in a sample would provide diagnosis of Alzheimer's disease.

Applicants respectfully disagree with the Examiner's position.

Although Applicants believe that the instant specification fully supports the claim that an isolated peptide consisting of amino acid residues 2-18 of SEQ ID NO:1 is diagnostic for Alzheimer's disease, in the interest of compact, efficient prosecution Applicants have amended the claims to recite that the isolated peptide is linked to Alzheimer's disease.

According to the web site dictionary.com the term "linked" refers to the condition of being associated with or connected to (see attached document as accessed from the internet; reference 1). The instant specification fully supports a connection and/or an association of the claimed peptide with Alzheimer's disease. The instant specification states at page 35, lines 14-18 that an objective of the invention is to evaluate samples containing a plurality of biopolymers for the presence of disease specific marker sequences which evidence a link to at least one specific disease state.

Claim 1 has been amended to specifically recite an isolated biopolymer marker consisting of amino acid residues 2-18 of SEQ ID NO:1. Claim 1, as amended herein, does not recite that the claimed

biopolymer marker is diagnostic for Alzheimer's disease, nor does it recite that the claimed isolated biopolymer marker is related to Alzheimer's disease, even though Applicants believe that the specification as originally filed fully supports both of these recitations. Furthermore, the phrase "consisting of" is closed language and excludes any element, step or ingredient not specified in the claim (see MPEP 2111.03). Thus, the scope of claim 1 is limited to this specific biopolymer marker (amino acid residues 2-18 of SEQ ID NO:1).

The Examiner is respectfully reminded that all questions of enablement are evaluated against the claimed subject matter (MPEP 2164.08) and that the claimed subject matter is interpreted in light of the specification. Thus, in the instant case, the specification should enable the evaluation of samples containing a plurality of biopolymer markers for the presence of disease specific marker sequences which evidence a link to at least one specific disease state; specifically the marker sequence amino acid residues 2-18 of SEQ ID NO:1 which evidences a link to Alzheimer's disease.

It has been established that in order to comply with the enablement requirement all that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the art (MPEP 2164.08).

Applicants assert that those of skill in the art are both

highly knowledgeable and skilled such that it is obvious that no undue experimentation would be required for a skilled artisan to follow any of the electrophoretic, chromatographic and mass spectrometric protocols presented in the instant specification in order to use the claimed invention. The Examiner agrees with Applicants' assertion (pages 3-4, final office action mailed on February 4, 2004). One of skill in the art would be able to view a gel, such as that shown in Figure 1 from which the claimed biopolymer marker was identified, and recognize a difference between two comparable samples (disease state vs. non-disease state) and further recognize that the peptides present within the gel are differentially expressed between the two sample types.

The Examiner provides an analysis of the data presented in Figure 1 which she asserts appears to be in conflict with Applicants' previous statements regarding the data.

Applicants stated in the previous Response filed on October 23, 2003 that the decreased presence of bands (for example, Band C2 which corresponds to Band C1) in samples obtained from Alzheimer's disease patients compared to samples obtained from patients age matched to the Alzheimer's disease patients represents the down-regulation and/or fragmentation of the proteins (including the claimed peptide) contained in these bands in Alzheimer's disease. However, the Examiner asserts that there appears to be no visible difference in intensity of Band C2 in sample 2 (AD sample),

samples 6 and 8 (normal age-matched control) and especially, sample 9 (pooled normal serum).

It can be observed, in Figure 1, that Bands C1 (age-matched) and C2 (AD) are not of the same intensity, and further that Band C1 is of greater intensity than corresponding Band C2. Thus, Applicants' previous statement regarding the down-regulation and/or fragmentation in Alzheimer's disease of proteins contained within Band C2 is accurate.

The Examiner concludes from her analysis of the data presented in Figure 1 that a skilled practitioner would not be able to distinguish between a normal sample (lane 9) and any of the AD samples.

Applicants respectfully disagree with the Examiner's conclusion.

Figure 1 shows a gel resulting from DEAE (anion-exchanging resin) column chromatography as carried out with a set of samples: 4 serum samples obtained from Alzheimer's disease patients (lanes 1-4, as read from the left), 4 serum samples obtained from patients age matched to the Alzheimer's patients (lanes 5-8, as read from the left) and 1 sample of serum pooled from a group of normal patients (lane 9, as read from the left). Bands which were observed to be differentially expressed between the disease (Alzheimer's disease) and non-diseased (age matched) states were excised from the gel for further analysis according to the methods of the

instant invention; for example, Band C1, seen in lane 5, patient serum sample AG-AD-H-S-002 was excised from the gel along with its corresponding band, Band C2, seen in lane 1, patient serum sample AD-H-S-004. Band C1 is present at a greater intensity in the samples obtained from patients age matched with the Alzheimer's patients (lanes 5-8) than in the samples obtained from Alzheimer's patients (Band C2, lanes 1-4). A band corresponding to Bands C1 and C2 is not seen in the sample obtained from normal patients (lane 9). This differential expression of the bands between samples is observable to one viewing the gel.

Thus, contrary to the Examiner's assertion, a skilled practitioner would be able to distinguish between a normal sample and an Alzheimer's disease sample. And further, Applicants contend that a skilled practitioner would find the data presented in Figure 1 adequate to suggest that the claimed peptide (amino acid residues 2-18 of SEQ ID NO:1) is linked to Alzheimer's disease.

The Examiner asserts that the specification, as originally filed, does not provide a precise protocol on how to analyze the data obtained by carrying out the disclosed protocol. Applicants respectfully disagree with the Examiner's assertion.

The "test of enablement" is whether one reasonably skilled in the art could make and/or use the invention from the disclosures in the patent combined with information known in the prior art without undue experimentation (see MPEP 2164.01).

Furthermore, the decision in *In re Brandstader* (179 USPQ 286; MPEP 2164.01) has established that the evidence provided by applicant (to overcome an enablement rejection) need not be conclusive but merely convincing to one of skill in the art.

Applicants respectfully submit that the instant specification provides sufficient evidence to convince one of skill in the art that the claimed peptide (amino acid residues 2-18 of SEQ ID NO:1) is linked to and/or associated with Alzheimer's disease.

Claim 1 has been amended to specifically recite an isolated peptide consisting of amino acid residues 2-18 of SEQ ID NO:1, a peptide which the instant specification identifies as related to Alzheimer's disease (page 46, lines 11-19). Claim 1, as amended herein, does not recite that the claimed isolated peptide is diagnostic for Alzheimer's disease, nor does it recite that the claimed isolated peptide is related to Alzheimer's disease, even though Applicants believe that the specification, as originally filed, fully supports both of these recitations. Furthermore, the phrase "consisting of" is closed language and excludes any element, step or ingredient not specified in the claims (see MPEP 2111.03). Thus, the scope of claim 1 is limited to this specific peptide (amino acid residues 2-18 of SEQ ID NO:1).

At page 46, lines 11-19 of the specification as originally filed, SEQ ID NO:1 is identified as having a molecular weight of about 1874 daltons. The description of Figure 2 at page 37

indicates that the spectra depicted in the figure is that of ion 1873. The spectra shown in Figure 2 was obtained from Band C1 as shown in the gel of Figure 1. The descriptions of the figures have been amended to clarify that the data shown in the figures is representative of the claimed peptide (amino acid residues 2-18 of SEQ ID NO:1) and the other disclosed peptides.

Figure 1 demonstrates that the claimed biopolymer marker peptide (amino acid residues 2-18 of SEQ ID No:1) shows increased intensity in samples obtained from patients age matched with the Alzheimer's patients when compared with samples obtained from the Alzheimer's patients. Thus, a difference is seen between two comparable samples (disease versus non-disease), suggesting that the differentially expressed peptide is linked to the disease state; in this instance, amino acid residues 2-18 of SEQ ID NO:1 are linked to Alzheimer's disease.

Applicants believe that the instant specification, as originally filed, discloses a precise protocol that one of skill in the art can carry out in order to practice the disclosed methods successfully. Page 5, line 9 to page 6, line 3 of the instant specification discloses a general outline of how to analyze the data obtained by carrying out the disclosed method. Page 26, lines 6-13 of the instant specification further describes how samples were compared to develop data and indicates how peptides were selected as notable sequences. This passage also discloses how

certain peptides were selected from a plurality of molecules found within a sample and how peptides were deemed to be evidentiary of a disease state. Page 47, lines 4-6 of the instant specification clearly states that the steps of the invention include obtaining a sample from a patient and conducting a MS analysis (mass spectrometry) on the sample. Thus, Applicants assert that the specification, as originally filed, provides a precise protocol on how to analyze the data obtained by the disclosed protocol.

Additionally, Applicants respectfully submit that such protocols are common practice in the field of proteomics. It is clear that the data presented in the instant specification was obtained by carrying out mass spectrometry. Thus, one of skill in this art would know how to analyze and obtain information from mass spectrometry profiles.

For example, Lubec et al. (see attached abstract Journal Neural Transmission Supplement 57:161-177 1999; reference 2) disclose an experiment in which proteomic techniques, specifically electrophoresis and mass spectrometry, were carried out to detect differences in protein expression between Down's syndrome patients, Alzheimer's patients and "normal" control patients. In a manner similar to that of the instant inventors, Lubec et al. analyzed the increase and/or decrease in expression of a particular protein (DRP-2) when hypothesizing about the neuropathological findings in Alzheimer's disease and Down's syndrome.

The data presented in the figures, derived from the working examples, discloses that the claimed peptide (amino acid residues 2-18 of SEQ ID NO:1) is differentially expressed between Alzheimer's disease and age matched controls, thus it can be reasonably predicted that such peptide is linked to Alzheimer's disease. Furthermore, the figures identify SEQ ID NO:1 and the specification discloses how such sequence was identified as a notable sequence in relation to Alzheimer's disease.

Thus, Applicants contend that a skilled practitioner would find that the data presented in the instant specification is convincing with regard to a link between the claimed biopolymer marker peptide (amino acid residues 2-18 of SEQ ID NO:1) and Alzheimer's disease.

Considering the above comments, it is clear that both the specification and the prior art disclose how to make and use the instant invention. Accordingly, Applicants respectfully contend that the instant invention passes the "test of enablement" since one skilled in the art could make or use the invention from the disclosures in the specification coupled with information known in the prior art without undue experimentation.

The Examiner asserts that Applicants' current reasoning appears to be in conflict with the Declaration of Lander under 37 CFR 1.132 filed on June 6, 2003, which presented information that the instant claimed biopolymer marker is not present in normal

human serum. The Examiner questions how one would distinguish AD samples from normal samples if both appear to have less of the biopolymer marker of SEQ ID NO:1, residues 2-18.

The process of distinguishing samples is described above. It is noted that the Declaration of Lander contains an inadvertent omission. The top profile in the figure is labeled as a profile obtained from the sera of an Alzheimer's patient, however the top profile is actually a profile obtained from a patient age matched to an Alzheimer's patient.

The Examiner asserts that the instant specification fails to present any description of samples used in experiments to determine the presence or absence of the claimed marker.

Applicants respectfully disagree with the Examiner's assertion and contend that the instant specification presents enough information about the samples used in carrying out the experiments. Line 17 of the protocol listed at page 41 of the instant specification clearly indicates that the sample used is sera. Page 47, line 5 of the instant specification indicates that the sample is obtained from a human patient. Page 48, lines 5-9 of the instant specification describes the types of samples which may be used in the methods/kits of the instant invention. Furthermore, the lanes of the gels in Figures 1, 3 and 5 are clearly labeled with patient numbers that indicate if the sample shown in each lane is obtained from an Alzheimer's patient, a patient age matched with an

Alzheimer's patient or a sample pooled from a plurality of normal patients. Thus, Applicants assert that there is enough information in the specification regarding the samples for one of skill in the art to determine the presence or absence of the claimed marker when carrying out the methods of the claimed invention.

The Examiner makes several assertions regarding the enablement of subject matter which is not claimed, including the assertion that the instant specification does not present information regarding presence or absence of the instant peptide (amino acid residues 2-18 of SEQ ID NO:1) in serum samples of pathological conditions other than Alzheimer's disease, or serum samples of patients suspected of having Alzheimer's disease, in which such marker would be present, followed up by a diagnosis of AD using other methods.

The Examiner is reminded that all questions of enablement should be evaluated against the claimed subject matter and the focus of the examination inquiry should be a question of whether everything within the scope of the claims is enabled (see MPEP 2164.08).

Accordingly, an Applicant is not required to enable material which is not claimed. The pending claims do not recite that the claimed peptide is diagnostic for any pathological condition, including Alzheimer's disease. Thus, no teachings regarding diagnostics are necessary in order to provide evidence for

enablement of the pending claims.

Additionally, Applicants assert that the intended purpose of the invention is to provide improved, alternative means for diagnosis of Alzheimer's disease which can easily be performed by an untrained individual without the need for additional testing. If "follow-up" diagnostic methods are required, then the diagnostic process is lengthened and the invention fails to fulfill its intended purpose.

The Examiner continues to maintain that the association of the claimed biopolymer marker with Alzheimer's disease is not supported by any evidence of record.

Applicants respectfully disagree with the Examiner and contend that one of skill in the art would understand that the claimed biopolymer marker is linked to Alzheimer's disease.

The guidelines for a "test of enablement" indicate that if a statement of utility in the specification contains within it a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated, 35 USC 112 is satisfied.

Additionally, it has been established that the mere fact that something has not previously been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it (see MPEP 2164.02).

The instant specification discloses a biopolymer marker (amino

acid residues 2-18 of SEQ ID NO:1) which is linked to Alzheimer's disease. The data derived from the working examples and presented in the figures clearly shows a link between the claimed biopolymer marker (amino acid residues 2-18 of SEQ ID NO:1)and Alzheimer's disease. A link between such specific peptide and Alzheimer's disease has not previously been shown.

The claimed biopolymer marker (amino acid residues 2-18 of SEQ ID NO:1) was found to be differentially expressed between Alzheimer's disease and age matched normal through use of proteomics techniques. The field of proteomics was established in the mid-1990's, is well-studied and is expanding rapidly to advance medical diagnostics and therapeutics. In proteomics research, differential protein expression patterns between normal and diseased cells are routinely analyzed and compared (see attached press release from Scimagix, Inc. as accessed from the internet; reference 3). Expression proteomics is the large-scale analysis of protein expression and function in which the goal is to detect and identify all-or a subset-of the proteins present in a particular sample and find out which of these proteins are present, absent or differentially expressed in a related sample subject to a specific variation (see attached document GIGA Proteomics Facility, Belgium as accessed from the internet; reference 4). Proteomics techniques make it possible for researchers to immediately highlight proteins that are differentially abundant in one state versus another (for

example, tumor vs. normal or before and after treatment; see attached article Liotta et al. Breast Cancer Research 2:13-14 1999 as accessed from the internet, page 2, second paragraph; reference 5). A protein found only in a diseased sample may prove to be a useful drug target or diagnostic marker (see attached document GIGA Proteomics Facility, Belgium as accessed from the internet; reference 4).

Although the prior art does not specifically recognize that the claimed peptide (amino acid residues 2-18 of SEQ ID NO:1) is related to Alzheimer's disease, it does recognize that when a peptide is identified in a body fluid sample from an Alzheimer's patient, it is immediately recognized as a potential diagnostic marker, even if the involvement of the peptide in the pathology of Alzheimer's disease in unknown. One of skill in the art would be familiar with this practice since it has been known in the art since at least 1992. See attached abstract of Gunnersen et al. (Proceedings of the National Academy of Science USA 89(24):11949-11953 1992; reference 6) in which the detection of glutamine synthetase in the cerebrospinal fluid of Alzheimer's disease patients lead to the suggestion of glutamine synthetase as a potential diagnostic biochemical marker. Thus, when one of skill in the art observes the claimed peptide identified in samples from an Alzheimer's disease patient or differentially expressed between Alzheimer's disease patients and non-diseased patients; one of

skill in the art would connect this peptide with potential diagnostics and/or therapeutics for Alzheimer's disease.

Applicants respectfully submit that since the specification demonstrates a link between the claimed peptide (amino acid residues 2-18 of SEQ ID NO:1) and Alzheimer's disease and such link connotes the use of the claimed peptide in potential diagnostics and/or therapeutics of Alzheimer's disease, the requirement of "how to use" under 35 USC 112, first paragraph is satisfied.

Thus, Applicants respectfully submit that one of ordinary skill in the art would find the suggestion of a link between the claimed peptide (amino acid residues 2-18 of SEQ ID NO:1) and Alzheimer's disease to be reasonable.

The claimed peptide is identified as a fragment of apolipoprotein J precursor protein at page 46, lines 11-19. Apolipoprotein J protein is also known as clusterin; see attached abstract of Moulson et al. (Journal of Cell Physiology 180(3):355-364 1999; reference 7). Clusterin (ApoJ) is a glycoprotein known to be expressed in response to tissue injury and has been associated with the pathology of Alzheimer's disease; see attached abstracts of Giannakopoulos et al. (Acta Neuropathology (Berl) 95(4):387-394 1998; reference 8) and BV Zlokovic (Life Science 59(18):1483-1497 1996; reference 9). It has been suggested that ApoJ exhibits an anti-amyloidogenic effect as it acts as a carrier protein of amyloid beta in body fluids, thus keeping it (amyloid

beta) in soluble form (BV Zlokovic Life Science 59(18):1483-1497 1996; reference 9) which is not deposited to form the amyloid plaques which are characteristic of Alzheimer's disease. It has also been suggested that low cellular expression of clusterin may be associated with the neuronal degeneration and death seen in Alzheimer's disease (Giannakopoulos et al. Acta Neuropathology (Berl) 95(4):387-394 1998; reference 8). A skilled artisan would find the data disclosed by the instant inventors to be reasonable since lack of ApoJ (of which the claimed biopolymer marker is a fragment) is associated with the pathology of Alzheimer's disease and the claimed biopolymer marker was found to be absent and/or show decreased expression in samples obtained from Alzheimer's disease patients. Thus, one of skill in the art would likely consider the claimed biopolymer to be linked to Alzheimer's disease.

The Declaration of Jackowski under 37 CFR 1.132 filed on October 23, 2003 provides additional clarification on how the claimed biopolymer marker was discovered to be absent and/or show decreased expression in serum samples of Alzheimer's disease patients and present in serum samples of patients age matched with the Alzheimer's disease patients. The experiments disclosed in the instant specification establish a mass spectrum profile with regard to the claimed biopolymer marker which is intended to be used as a reference point for comparison to an unknown sample in a

diagnostic assay. The mass spectrum profile of the claimed biopolymer marker (amino acid residues 2-18 of SEQ ID NO:1) is characterized by a peak at around 1874 daltons (see page 46, lines 11-14 and Figure 2). This known mass spectrometric profile of the claimed biopolymer marker is compared with the mass spectrometric profile of the unknown sample in the diagnostic assay contemplated by the instant inventors.

Additionally, the Examiner asserts that a definitive diagnosis of Alzheimer's disease could only be made during postmortem examination or at brain biopsy.

The Examiner is reminded that the mere fact that something has not previously been done clearly is not, in itself, a sufficient basis for rejecting all applications purporting to disclose how to do it (MPEP 2164.02).

The conventional "definitive diagnosis" does not control the usefulness of other methods suggested for diagnosis. Diagnostic methods other than postmortem examination and brain biopsy have been deemed valuable for diagnosing Alzheimer's disease. For example, Applicants submit their own patent, US 6,451,547 B1 (Jackowski et al.; reference 10) which claims methods for diagnosing Alzheimer's disease by detecting the presence of biochemical markers in bodily fluid.

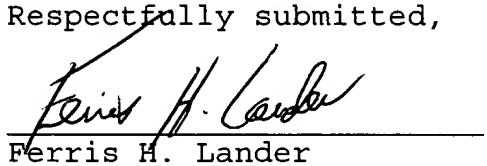
In conclusion, Applicants claim that the absence and/or decreased expression in samples obtained from Alzheimer's disease

patients of a biopolymer marker consisting of amino acid residues 2-18 of SEQ ID NO:1 and presence of this biopolymer marker in samples obtained from patients age matched with the Alzheimer's disease patients is indicative of a link to Alzheimer's disease; a statement which is enabled by the instant specification, as evidenced by the arguments presented herein. Applicants respectfully submit that one of ordinary skill in the art when reviewing the instant specification, given the level of knowledge and skill in the art, would recognize how to use the claimed biopolymer marker as a marker for Alzheimer's disease. Thus, Applicants respectfully request that this rejection under 35 USC 112, first paragraph now be withdrawn.

CONCLUSION

In light of the foregoing remarks, amendments to the specification and amendments to the claims, it is respectfully submitted that the Examiner will now find the claims of the application allowable. Favorable reconsideration of the application is courteously requested.

Respectfully submitted,



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ink[1] Pronunciation Key (lĭngk)

1. One of the rings or loops forming a chain.
2.
   a. A unit in a connected series of units: *links of sausage; one link in a molecular chain.*
   b. A unit in a transportation or communications system.
   c. A connecting element; a tie or bond: *grandparents, our link with the past.*
3.
   a. An association; a relationship: *The Alumnae Association is my link to the school's present administration.*
   b. A causal, parallel, or reciprocal relationship; a correlation: *Researchers have detected a link between smoking and heart disease.*
4. A cuff link.
5. *Abbr.* **li** A unit of length used in surveying, equal to 0.01 chain, 7.92 inches, or about 20.12 centimeters.
6. A rod or lever transmitting motion in a machine.
7. *Computer Science.* A segment of text or a graphical item that serves as a cross-reference between parts of a hypertext document or between files or hypertext documents. Also called **hotlink, hyperlink**.

linked, link·ing, links
tr.

1. To connect with or as if with a link: *linked the rings to form a chain.*

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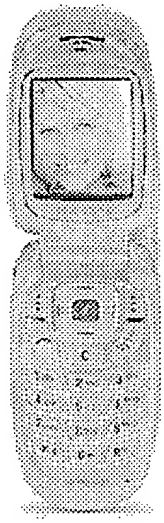

cingular

Certain restrictions apply. 2 yr. agreement required.

See Synonyms at join.
2. *Computer Science.* To make a hypertext link in: *linked her webpage to her employer's homepage.*

v. intr.

1. To become connected with or as if with a link: *The molecules linked to form a polymer.*
2. *Computer Science.* To follow a hypertext link: *With a click of the mouse, I linked to the company's website.*

[Middle English `linke`, *of Scandinavian origin*; akin to Old Norse `hlekkr, *hlenkr`, from *`hlenkr`.]

nk'er *n.*

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nked [P] Pronunciation Key (lĭngkt)
adj.

1. Connected, especially by or as if by links.
2. *Genetics.* Exhibiting linkage.
3. *Computer Science.* Provided with links.

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nked (lĭngkt)
adj.

Exhibiting linkage.

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Main Entry: **linked**
Pronunciation: `'li[ng](k)t`
Function: *adjective*

marked by linkage and especially genetic linkage <*linked* genes>

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Linked

adj : connected by a link, as railway cars or trailer trucks [syn: coupled, joined]

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Linked

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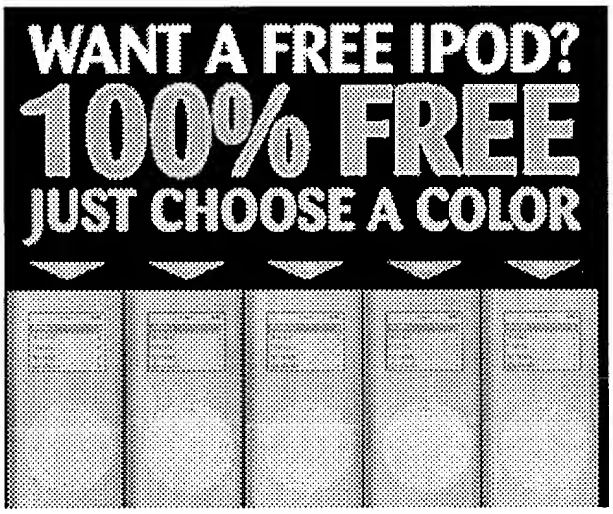

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Expression of the dihydropyrimidinase related protein 2 (DRP-2) in Down syndrome and Alzheimer's disease brain is downregulated at the mRNA and dysregulated at the protein level.

Lubec G, Nonaka M, Krapfenbauer K, Gratzer M, Cairns N, Fountoulakis M.

Department of Pediatrics, University of Vienna, Austria. gert.lubec@akhwien.ac.at

Deteriorated migration, axonal pathfinding and wiring of the brain is a main neuropathological feature of Down Syndrome (DS). Information on the underlying mechanisms is still limited, although basic functions of a series of growth factors, cell adhesion molecules, guidance factors and chemoattractants for brain histogenesis have been reported. We used proteomics to detect differences in protein expression between control, DS and Alzheimer's disease brains: In five individual brain regions of 9 individuals of each group we performed two dimensional electrophoresis with MALDI--identification of proteins and determined mRNA levels of DRP-2. Significantly decreased mRNA levels of DRP-2 in four brain regions of patients with DS but not with AD as compared to controls were detected. 2D electrophoresis revealed variable expression of DRP-2 proteins, which showed a high heterogeneity per se. Dysregulation of DRP-2 was found in brains of patients with DS and AD presenting with an inconsistent pattern, which in turn may reflect the inconsistent neuropathological findings in patients with DS and AD. The decrease of mRNA DRP-2 steady state levels in DS along with deteriorated protein expression of this repulsive guidance molecule of the semaphorin/collapsin family, may help to explain deranged migration and histogenesis of DS brain and wiring of AD brain.

PMID: 10666674 [PubMed - indexed for MEDLINE]

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Scimagix and Compugen Sign Re-sale Agreement for 2D-Gel Analysis Product

Creating High-throughput 2D-Gel Analysis and Mining Products for Proteomics

REDWOOD SHORES, Calif., February 21, 2001 — Scimagix™ Inc. today announced an agreement for Scimagix to resell Compugen's Z3™ automated system for rapid analysis of 2-dimensional electrophoresis (2D) gels. The combination of Z3 with Scimagix's SIMS™ image data management system and ProteinMine™ 2D-gel analysis and mining application provides researchers with robust and accurate comparisons of 2D gels for differential protein expression and high-speed searching of similar gel expression patterns, and the ability to correlate 2D-gel data with other image or non-image data.

Under the agreement, Scimagix will resell Compugen's Z3 product worldwide together with Scimagix' SIMS product; both companies plan to integrate their products for seamless exchange of data. Financial details were not disclosed.

"Our agreement combines Compugen's extensive gel analysis methods with Scimagix's unique capability to search protein expression patterns," said Robert Dunkle, president and CEO of Scimagix. "Together we can offer the best approach for high-throughput 2D-gel analysis and mining, enabling researchers to derive faster and better insights from the information found in 2D gels. This high-throughput approach to analysis and mining of 2D gels is expected to accelerate proteomics research and drug discovery."

"Through our relationship with Scimagix we are able to offer customers a stronger proteomics solution, therefore serving the market in a more complete manner," said Dr. Michal Preminger, Vice President, Proteomics Business, Compugen. "Our agreement will expand the reach of both companies, providing innovative solutions for image data analysis and mining for pharmaceutical R&D."

Z3 Automated 2D-Gel Analysis
Compugen's Z3 product sets a benchmark for rapid and visually-oriented comparison of 2D gels to identify differentially expressed proteins, reducing the average overall analysis time per gel-pair to less than 15 minutes. In proteomics research, differential protein expression patterns between normal and diseased cells are routinely analyzed and compared. Z3 automates this process of comparative proteomics analysis to determine those proteins that are differentially expressed. Z3 is able to accomplish this through the use of complex image-analysis algorithms that rapidly and accurately align two gels, resulting in an easy-to-read visual analysis.

For information regarding any of these press releases, please contact:

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ProteinMine™ High-throughput Mining of Protein Patterns
Scimagix's ProteinMine 2D-gel analysis and mining application, the first software to search scientific images by visual content, offers researchers an approach that complements the Z3 analysis system. Scimagix's proprietary technology enables the enumeration, quantitation and identification of proteins and protein patterns across thousands of gels, along with the ability to search on any combination of changes such as protein up-regulation, down-regulation or no change. Gels recovered by a search can be used to help researchers explore and better understand mechanisms of action and operative pathways, and can be combined with other image and non-image data through SIMS. The open architecture of SIMS enables Scimagix to work in collaboration with Compugen and other vendors to exchange and integrate data.

About Compugen
Compugen (Nasdaq: CGEN) develops and markets platforms, tools and products that accelerate post-genomic research, the advanced study of proteins and protein pathways, and drug-target discovery. These products and services include: LEADS, Gencarta, DNA Chip design, Z3, LabOnWeb.com and Bioaccelerators. Compugen's in-house molecular biology laboratories provide validation of its methodologies and also conduct original genomic and proteomic research. For additional information, please visit Compugen's corporate Web site at www.cgen.com and the company's Internet research engine for molecular biologists at www.LabOnWeb.com.

About Scimagix Inc.
Scimagix™ Inc., based in Redwood Shores, Calif., is a leading provider of image informatics solutions for the pharmaceutical and biotechnology industries. Its SIMS™ - Scientific Image Management System and ProteinMine™ 2D-gel analysis and mining application for proteomics research are the first in a new class of image retrieval, analysis and mining software that allows researchers to derive added value from images. Scimagix's products serve five of the top 15 pharmaceutical companies, including Pfizer Global R&D and Eli Lilly & Co. Unlocking the value of scientific images, image informatics promises to become an essential and pervasive technology for gaining new insights and direction for discovery research and development.

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What is proteomics?

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The completion of the Human Genome Sequencing Project represents a major achievement in modern science. The wealth of information obtained through human genome analysis will certainly increase our knowledge of the cell biochemistry that defines the boundary between a healthy and a diseased individual. It will also contribute to the development of new tools for the diagnosis and treatment of human diseases.

Today, in terms of DNA sequences, scientists have in hand the complete genomes of a wide variety of organisms, spanning all forms of life from viruses, phages, archaea, and bacteria to eukaryotes.

Considering the size of the human genome (~3,200 Mb), an unexpectedly small number of human genes has been predicted: between 20,000 and 35,000 (the precise number is still the subject of much controversy). Genes make up less than 2 percent of the human DNA; the remaining DNA has important but as yet unknown functions that may include regulating genes and maintaining chromosome structure.

Direct access to the genome, however, is only a preliminary step towards understanding biological processes, because detecting all coding regions in a genome sequence remains a difficult task. This is especially true in eukaryotes, where current algorithms, although quite efficient, are unable to detect with certainty all exons, are ill-equipped to discriminate different splice variants, and are unable to identify small proteins (which are numerous and essential to many biological processes).

Even if we identify all potential protein coding regions in the human genome, we will still be missing some crucial information, because genomic information by itself does not allow efficient prediction of all the post-modifications observed in proteins.

Diverse mechanisms can result in the expression of many protein variants from the same gene locus in a single species: single nucleotide polymorphisms (SNP), gene splicing, alternative splicing of pre-mRNA, RNA editing, translational frame shifts and hopping, proteolytic cleavage of the protein (to eliminate signal sequences or to create transit peptides or pro-peptides) and post-translational modifications of amino acid residues which affect a vast majority of proteins (acetylation, phosphorylation, glycosylation, lipidation, etc - more than a hundred different types of PTMs are currently known).

Hence, the number of different protein molecules expressed by the human genome is probably closer to a million than to the hundred thousand generally considered by genome scientists.

The **"proteome"** can be defined as all proteins expressed by a cell at a particular time and under specific conditions. The aim of **"proteomics"** is to identify, characterise, and quantify all proteins involved in a particular pathway, organelle, cell, tissue, organ, or organism and that can be studied simultaneously in order to obtain accurate and comprehensive data about that system and to correlate expression-level changes and/or protein PTMs with growth conditions, the cell cycle stage, a disease state, external stimuli, levels of expression of other proteins, or other variables.

Why proteomics?

Proteomics has the potential to revolutionise the development of innovative clinical diagnostics and pharmaceutical therapeutics.

There are many reasons why understanding the proteome will be more useful than understanding the genome:

- Whereas every cell in an organism contains an identical copy of the complete set of genes necessary to build a functional individual, this set of genes is only a source of information, which must be expressed in order to function. In complex organisms, this information is used differently in different cells in order to produce different types of tissues, organs, or cells (i.e. liver, muscle, bone, neurons, blood cells...), and these differences are due to the proteins that exploit the genetic information differently in each cell.

What is proteomics?

- From these considerations it appears that the real actors behind the complexity of life-sustaining biochemical mechanisms are the proteins, with their intricate patterns of interactions with each other and with other biological molecules and their relations with the external environment.

- Whilst some protein polymorphisms are linked to disease states, most are not. Yet they do have in many cases a direct or indirect effect on the activities of the proteins concerned. For example, it is estimated that each human protein exists, on the average, in ten to fifteen different post-translationally modified forms, with - presumably - different functions. Much of the information processing in healthy and diseased human cells can be studied only at protein level, and there is increasing evidence linking minor changes in expression of some modifications with specific diseases.

- While some disorders are known to result from a single gene defect, such as cystic fibrosis (chromosome 7) and sickle cell anaemia (chromosome 11), it is generally accepted that many common diseases such as diabetes, hypertension, deafness, and cancers have more complex causes that may be a combination of sequence variations in several genes - perhaps 20 or many more - on different chromosomes, in addition to environmental factors. It is not possible to identify these genes by sequencing the genome(s) of one, two, or even ten different people – but one can study the proteomes of these individuals to select which 20 or so genes are the important ones.

- In many human diseases, what leads to disease is an incorrect modification or conformation of a normal protein (for example, in protein-folding-related diseases like Alzheimer's, Parkinson's, new-variant CJD, and type II diabetes). Such modifications cannot be seen in or deduced from the genome.

- From a practical standpoint, proteins are almost always useful for disease diagnosis, and the targets of nearly all drugs used in disease therapy are proteins. In order to design the most efficient drug for any disease, one has to find the right target. The best way to do this is to determine all the forms that an individual protein can take, all the proteins with which it interacts, and all the pathways in which it participates.

How do we study a proteome?

Although DNA micro-arrays enable us to view a genome-wide number of active gene products simultaneously in the form of mRNAs, there is often no direct relationship between the *in vivo* concentration of an mRNA and the level of its encoded protein. Differential rates of mRNA translation into protein and differential rates of protein degradation *in vivo* are two factors that confound the extrapolation of mRNA levels to protein expression profiles. Additionally, micro-array analysis is unable to detect, identify, or quantify post-translational protein modifications, which often play a key role in modulating protein function.

Proteomics comprises all comprehensive, high-throughput methods enabling us to display and identify the largest possible number of proteins in a proteome, and to determine how they relate to each other though changes in expression levels or PTMs in response to specific variations in the environment or according to the state of the system under study (i.e. organ, tissue, cell, organelle, micro-organism, or protein complex).

The various techniques used to study the proteome are not as straightforward as those used in transcriptomics, and they span various aspects of protein function:

Structural proteomics is the large-scale analysis of protein structures.
This is achieved using technologies such as high-throughput automated protein expression systems combined with X-ray crystallography and NMR spectroscopy. Structural proteomics also includes extensive *in silico* comparisons and analyses of protein primary and tertiary structures deposited in the

What is proteomics?

various databases or deduced from genome sequences, with a view to exploring common structural motifs and how they relate to diverse protein functions. Structural analysis can contribute to identifying the functions of newly discovered genes or to showing where drugs bind to proteins or where proteins interact with each other.

Interaction proteomics is the large-scale analysis of protein interactions.
One of the best ways to determine the function of a newly discovered protein is to identify with which molecules it interacts or associates. All classical protein isolation and fractionation techniques (centrifugation, chromatography...) and other technologies such as tandem affinity purification, mass spectrometry, phage display, and the yeast two-hybrid system can be used to isolate protein complexes (for example membrane translocation complexes, ribosomal complexes, transcriptome, spliceosome, nucleosome, respiratory, or photosynthetic complexes) in order to determine protein functions and to study how and why proteins assemble into larger complexes.

Expression proteomics is the large-scale analysis of protein expression and function.
The goal here is to detect and identify all - or a subset - of the proteins present in a particular sample (e.g. a cell, a bacterium, an organelle, or an isolated protein complex) and find out which of these proteins are present, absent, or differentially expressed in a related sample subject to a specific variation. A protein found only in a diseased sample may prove to be a useful drug target or diagnostic marker ("biomarker"). Methods such as two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) or multidimensional liquid chromatography are generally used to separate proteins or peptides in a complex mixture. Following separation, proteins are identified by mass spectrometry combined with protein database searches carried out with appropriate software algorithms.

Protein and antibody micro-arrays are still under development. They may hold enormous potential for proteomic studies. At present, however, their use is far from widespread, and some technological details remain to be dealt with before they become a robust and reliable platform for research and diagnostics.

One of the main challenges encountered in proteomic studies is due to the huge dynamic range of protein expression. In human plasma, for instance, 10 orders of magnitude in concentration separate albumin from the rarest proteins now measured clinically. The difference is expected to reach 12 orders of magnitude in certain proteomes. Under such circumstances high-abundance proteins, sometimes referred to as "housekeeping" proteins, can severely interfere with the detection and profiling of proteins present in low abundance, which are often the interesting ones to study (i.e. transcription factors, kinases, membrane receptors...).

The preparation of a well-defined proteome sample is the basis of any successful proteomic study. Problems arise from the difficulty in preparing or displaying a sample representative of a chosen proteome because of the inherent characteristics of some proteins (poor extraction and/or solubilisation of hydrophobic membrane proteins, very acidic or basic proteins, very large or small proteins).

These considerations drive the effort to design novel proteomics instrumentation and methodology. First, the problem of sample complexity can be addressed by the use of defined reagents and extraction methods and specialised prefractionation techniques for isolating a particular proteome subset. Secondly, increased sensitivity and a wide dynamic range are particularly important for the instrumentation used in detection.

Despite these challenges, proteomics has a tremendous contribution to make towards understanding biological functions and designing better drugs and diagnostics. It is thus expected to drive much of the growth in life science research and instrumentation in the next 5 to 10 years.

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Beyond the genome to tissue proteomics

Lance A Liotta ✉ and Emanuel F Petricoin
National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Breast Cancer Res 1999, **2**:13-14 doi:10.1186/bcr23

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Full text

The ongoing revolution in molecular medicine can be divided into three phases. The first phase is gene discovery, in which the tools of molecular biology are applied to identify and sequence previously unknown genes. Identification of most of the expressed human genes will be accomplished before 2005. The second phase is molecular fingerprinting, which correlates the genomic state, the complementary DNA expression pattern, and the protein repertoire with the functional status of the cells or tissue. The promise of this phase is that expression profiles can uncover clues to functionally important molecules, and will generate information to tailor a treatment to the individual patient. The third phase is the synthesis of proteomic information into functional pathways and circuits in cells and tissues. This must take into account the dynamic state of protein post-translational modifications and protein-protein or protein-DNA interactions. Through an integrated genomic/proteomic analysis, the ultimate outcome will be an actual functional understanding of the molecular events that underlie normal development and disease pathophysiology. This higher level of functional understanding will be the basis for true rational therapeutic design.

Progress in these three phases of molecular medicine is largely driven by new technologies. The development of polymerase chain reaction, high throughput sequencing, and bioinformatics has been a driving force in the first phase. In the second phase, microhybridization arrays applied to genetic analysis and gene expression [1] is a powerful new tool that has entered the commercial sector, and is becoming widely available to researchers. As more genes are identified, it is likely that specialized arrays will be offered that are specific for a tissue type (eg mammary gland chip), physiologic process (eg apoptosis chip, angiogenesis chip, invasion chip) or class of genes (eg suppressor gene chip, oncogene chip).

Whereas DNA is an information archive, proteins do all the work of the cell. The existence of a given DNA sequence does not guarantee the synthesis of a corresponding protein [2,3]. The DNA sequence is also not sufficient to describe protein structure, function, and cellular location. This is because protein complexity and versatility stems from context-dependent post-translational processes such as phosphorylation, sulfation, and glycosylation. Moreover, the DNA code does not provide information about how proteins link together into networks and functional machines in the cell. In fact, the activation of a protein signal pathway causing a cell to migrate, die, or initiate division can immediately take place before any changes occur in DNA/RNA gene expression. Consequently, the technology to drive the molecular medicine revolution into the third phase is emerging from protein analytic methods.

The term 'proteome', which denotes all the proteins expressed by a genome, was first coined in late 1994 at the Siena two-dimensional gel electrophoresis meeting [4]. Proteomics is proclaimed as the next step after genomics. A goal of investigators in this exciting field is to assemble a complete library of all of the proteins. Only a small percentage of the proteome has been cataloged to date [2,3]. Because 'polymerase chain reaction for proteins' does not exist, sequencing the order of 20 possible amino acids in a given protein remains relatively slow and labor intensive, compared with nucleotide sequencing. Although a number of new technologies are being introduced for high throughput protein characterization and discovery [3,5], the mainstay of protein identification continues to be two-dimensional gel electrophoresis. Two-dimensional electrophoresis can separate proteins by molecular weight in one dimension and charge in the second dimension. When a mixture of proteins is applied to the two-dimensional gel, individual proteins in the mixture are separated out into signature locations on the display, depending on their individual size and charge. Each signature is a 'spot' on the gel, which can constitute a unique single protein species. The protein spot can be procured from the gel and a partial amino acid sequence can be read. In this manner known proteins can be monitored for changes in abundance under treatment or new proteins can be identified. An experimental two-dimensional gel image can be captured and overlayed digitally with known archived two-dimensional gels. In this way it is possible to immediately highlight proteins that are differentially abundant in one state versus another (eg tumor versus normal, or before and after hormone treatment).

Two-dimensional gels have traditionally required large amounts of protein starting material, equivalent to millions of cells. Thus, their application has been limited to cultured cells or ground-up heterogeneous tissue. Not unexpectedly, this approach does not provide an accurate picture of the proteins that are in use by cells in real tissue. Tissues are complicated structures composed of hundreds of interacting cell populations in specialized spatial configurations. The fluctuating proteins expressed by cells in tissues may bear little resemblance to the proteins made by cultured cells that are torn from their tissue context and reacting to a new culture environment. Proteins extracted from ground-up tissue will represent an averaging-out of proteins from all of the heterogeneous tissue subpopulations. For example, in the case of breast tissue the glandular epithelium constitutes a small proportion of the tissue; the vast majority is stroma and adipose. Thus, it has previously been impossible to obtain a clear snapshot of gene or protein expression within normal or diseased tissue cell subpopulations.

To address the tissue-context problem, new technology is again coming to the rescue; creating 'tissue proteomics' as an exciting expanding discipline. Two major technologic approaches have been successfully used to sample macromolecules

directly from subpopulations of human tissue cells. The first technology is laser capture microdissection. This is a technology for procuring specific tissue cell subpopulations under direct microscopic visualization of a standard stained frozen or fixed tissue section on a glass microscope slide. This technology was invented at the US National Institutes of Health and is commercially available through Arcturus Engineering (Mountain View, CA, USA; www.arctur.com). Tissue cells procured by laser capture microdissection have been used for highly sensitive and reproducible proteomic analysis using two-dimensional gels and other analytic methods [6,7,8].

A second major approach to isolate tissue cell subpopulations is affinity cell sorting of dissaggregated cells from pieces of fresh tissue. A highly notable application of this technology in the field of breast physiology was recently reported [9] in a study resulting from a collaboration between Oxford Glycosciences (Oxford, UK) and the Ludwig Institute (London, UK). In that study the investigators separated and purified normal human breast luminal and myoepithelial tissue from reduction mammoplasty specimens using double antibody magnetic affinity cell sorting and Dynabead magnetic sedimentation (Dynal Inc, UK). After using enzymatic treatments and various incubation, separation, and washing steps, the investigators obtained purified luminal and myoepithelial cells in yields of 5×10^6-2×10^7. Proteins from these cell populations were then analyzed using two-dimensional gels. A master image for each cell type comprising a total of 1738 distinct proteins was derived. The investigators found 170 protein spots that were elevated twofold or more between the two populations. Of these, 51 were further characterized by tandem mass spectroscopy. The proteins preferential to the myoepithelial cells contained muscle-specific enzymes and structural proteins consistent with the contractile muscle-related derivation of these cell types.

Myoepithelial cells are a fascinating component of breast tissue. They are thought to play important roles in duct and lobule growth, matrix architecture, and remodeling after lactation and involution. A pathologic hallmark of early cancer progression from carcinoma *in situ* to invasive cancer is the loss or redistribution of myoepithelial cells. The conspicuous absence of myoepithelial cells in breast cancer progression could mean that these cells produce suppressor proteins that normally keep the malignant cells in check. Thus, one or more of the proteins identified in the study by Page *et al* [10] could be candidate cancer prevention molecules. The authors of that study concluded that 'These observations demonstrate that proteomics has the refinement and sensitivity to find proteins that are either uniquely or differentially expressed between different cell types, the consequences of which could enable new strategies for drug discovery.'

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Detection of glutamine synthetase in the cerebrospinal fluid of Alzheimer diseased patients: a potential diagnostic biochemical marker.

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In this report, 8- and 2-azidoadenosine 5'-[gamma-32P]triphosphate were used to examine cerebrospinal fluid (CSF) samples for the presence of an ATP binding protein unique to individuals with Alzheimer disease (AD). A 42-kDa ATP binding protein was found in the CSF of AD patients that is not observed in CSF from normal patients or other neurological controls. The photolabeling is saturated with 30 microM 2-azidoadenosine 5'-[gamma-32P] triphosphate. Photoinsertion can be totally prevented by the addition of 25 microM ATP. Photoinsertion of 2-azidoadenosine 5'-triphosphate into the protein is only weakly protected by other nucleotides such as ADP and GTP, indicating that this is a specific ATP binding protein. A total of 83 CSF samples were examined in a blind manner. The 42-kDa protein was detected in 38 of 39 AD CSF samples and in only 1 of 44 control samples. This protein was identified as glutamine synthetase [GS; glutamate-ammonia ligase; L-glutamate:ammonia ligase (ADP-forming), EC 6.3.1.2] based on similar nucleotide binding properties, comigration on two-dimensional gels, reaction with a polyclonal anti-GS antibody, and the presence of significant GS enzyme activity in AD CSF. In brain, GS plays a key role in elimination of free ammonia and also converts the neurotransmitter and excitotoxic amino acid glutamate to glutamine, which is not neurotoxic. The involvement of GS, if any, in the onset of AD is unknown. However, the presence of GS in the CSF of terminal AD patients suggests that this enzyme may be a useful diagnostic marker and that further study is warranted to determine any possible role for glutamate metabolism in the pathology of AD.

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Clusterin (Apo J) regulates vascular smooth muscle cell differentiation in vitro.

Moulson CL, Millis AJ.

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Previously we reported a significant and substantial increase in the synthesis and secretion of clusterin in cultured porcine vascular smooth muscle cells (VSMC) during the time when the VSMC culture modulates from a proliferating monolayer morphology to a nodular cell culture morphology. That in vitro process appears to recapitulate some aspects of in vivo vascular remodeling in response to injury and is facilitated by the presence of a well-developed extracellular matrix. To directly test the hypothesis that clusterin regulates VSMC phenotypic modulation, cultured VSMC were stably transfected with an expression plasmid containing the full-length murine clusterin sequence in antisense orientation. Twenty-four clones were selected on the basis of neomycin resistance and characterized for clusterin expression and culture morphology. In contrast to clone SM-CLU18AS, which expresses a high level of clusterin and forms multicellular nodules, clone SM-CLU13AS expresses a low level of clusterin and does not form nodules even in the presence of a preformed collagen gel. Importantly, clusterin-negative SM-CLU13AS retains the ability to form nodules in an environment containing exogenous clusterin. SM-CLU13AS forms nodules when cultured in Matrigel (which contains clusterin) and in the presence of clusterin-containing conditioned media prepared from nodular SMC cultures or SM-CLU18AS cultures. These results demonstrate that clusterin is required for VSMC nodule formation and suggest that it may play a role in smooth muscle cell reorganization in the vascular wall.

PMID: 10430175 [PubMed - indexed for MEDLINE]

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1: Acta Neuropathol (Berl). 1998 Apr;95(4):387-94.

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Possible neuroprotective role of clusterin in Alzheimer's disease: a quantitative immunocytochemical study.

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Clusterin is a secreted glycoprotein that is expressed in response to tissue injury both in peripheral organs and in the brain. Recent studies have shown a substantial increase in clusterin mRNA in pyramidal neurons of the hippocampus and the entorhinal cortex in Alzheimer's disease (AD), with clusterin immunoreactivity occurring in neuropil threads, neurofibrillary tangles (NFT), and senile plaques. To elucidate further the role of this protein in the degenerative process, a quantitative study of its distribution in the cerebral cortex of non-demented and AD patients, all older than 85 years of age, was performed using immunocytochemistry. Using a stereological approach, we found that in cortical areas affected in AD, such as the entorhinal, inferior temporal and superior frontal cortices, the percentage of NFT-free neurons displaying clusterin immunoreactivity was significantly higher than that in non-demented cases. No such increase in the density of clusterin-immunoreactive neurons was seen in cortical areas that were less affected in the disease process. Furthermore, clusterin immunoreactivity was rarely observed in NFT-containing neurons. In conjunction with previous observations in peripheral tissues, these data suggest that clusterin may have a neuroprotective role, and that in AD, low cellular expression of this protein may be associated with neuronal degeneration and death.

PMID: 9560017 [PubMed - indexed for MEDLINE]

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1: Life Sci. 1996;59(18):1483-97.

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CANCER GENOME ANATOMY PROJECT

Cerebrovascular transport of Alzheimer's amyloid beta and apolipoproteins J and E: possible anti-amyloidogenic role of the blood-brain barrier.

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It is uncertain whether soluble circulating amyloid beta (sA beta) is the precursor of amyloid beta (A beta) found in cerebrovascular and parenchymal amyloid lesions in Alzheimer's Disease, and if so, how the transition to the filamentous form is brought about. Several lines of evidence suggest that apolipoprotein E (apoE) and apolipoprotein J (apoJ) may be involved in the regulation of amyloidogenesis. They both bind sA beta/A beta in vivo and in vitro. It has been suggested that apoE may modulate beta-pleated conformation of A beta and therefore act as a proamyloidogenic factor. On the other hand, apoJ as a major carrier protein of sA beta in body fluids may keep the peptide in a soluble form, thus having an anti-amyloidogenic effect. Using a well established guinea-pig brain perfusion model we have studied the blood-brain barrier (BBB) processes involved in the regulation of cerebral capillary sequestration, transport and metabolism of i) sA beta 1-40 and sA beta 1-42, synthetic peptides identical to the 40 and 42 residue forms of A beta, found primarily in vascular deposits and senile plaques, respectively; and ii) apoJ, apoE3 and apoE4 alone, and in a complex with sA beta. Specific saturable BBB luminal binding of both peptides was followed by transport into brain parenchyma and metabolism at the abluminal side of the BBB and/or in brain. The capillary sequestration of sA beta 1-40 was significant, while retention by the microvasculature of sA beta 1-42 was negligible. Binding to microvessels and blood-to-brain transport of both intact apoJ and sA beta 1-40 apoJ complexes were among the highest ever recorded for peptides and proteins at the BBB in vivo. These processes appear to be mediated by glycoprotein 330 (gp330/megalin), a receptor for multiple ligands, including apoJ. In contrast, capillary retention and transport of apoE3, apoE4 and sA beta 1-40-apoE3 complex were low to negligible, while blood-brain transport of sA beta 1-40-apoE4 was moderate. It is suggested that normal BBB may have predominantly anti-amyloidogenic functions by i) degrading sA beta during blood-to-brain transport; ii) favoring sequestration and transport of apoJ alone and in complex with sA beta via gp330 receptor-mediated mechanism and iii) excluding apoE3 and apoE4 isoforms from brain.

Publication Types:

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